Project Specific (Special) Protocols

***Acetobacter* Media Recipe** 5-28-14 (Source)

For 500mL of media add:

10g Glucose

2.5g Tryptone (or peptone)

2.5g Yeast Extract

1.35g Na_2HPO_4

0.75g Citric Acid

7.5g Agar (if making plates)

500mL Water

***Acetobacter* Transformation** 7-17-14

1. Follow general electroporation protocol from: http://2013.igem.org/wiki/images/5/5c/The_iGEMer%E2%80%99s_Guide_to_the_Galaxy_%28Stanford-Brown%29.pdf
2. There are a very few aliquots of electrocompetent cells in box in the 2nd (maybe 3rd) to bottom shelf of -80C freezer. On the left hand side of this shelf, there are 4 15mL falcon tubes stuck directly into the freezer. These are more electrocompetent cells! just aliquot them. (40 microL)
3. Use 2500 V for electroporation.
4. Use 5 microL plasmid mix (I have no justification for this, but it works)
5. Fill cuvette to 100 microL. This usually means 40 cells + 5 plasmid + 55 water. To avoid heating, I normally add chilled water and plasmid to cell aliquot tube, then transfer everything to cuvette.
6. Keep cuvette, and the black piece of the electroporator that holds the cuvette, in fridge right before use.
7. Avoid moisture on cuvette surface! you might want to very gently and quickly (to avoid heating) go over cuvette with paper towel right before electroporation
8. Incubate in SOC at RT, rather than 37 C. Do this for ~2 hrs, rather than 1 hr.

Radiation Assay 9-2-14

1. Grow an overnight culture in LB + Antibiotic

2. In the morning, take 50 uL of culture and add to 2 mL of LB + antibiotic media.

3. Incubate 2-4 hours at 37C and aim to stop incubation when OD is 0.6-0.8

4. Take 1 mL and place in Eppendorf tube. Spin down at 8000 rpm for 3 min.

5. Aspirate LB and resuspend in 1mL of 0.9% NaCl.

6. Fill 6 tubes with 900 uL of NaCl solution. Serially dilute the cells 1:10 (100 uL).

7. Drop 10 uL of each dilution in the first dosage row.

8. Expose to UV radiation (3 J/mˆ2).

9. Do this for each row. The rows previously exposed to UV will get a cumulative dose until finally you reach the control row which receives no radiation.

Site-directed mutagenesis 7-9-14

Component	Volume	Final Concentration
Q5 High-Fidelity 2X Master Mix	12.5 µl	1X
10 µM Forward mutagenesis primer	1.25 µl	0.5 µM
10 µM Reverse mutagenesis primer	1.25 µl	0.5 µM
DpsMP1 or MntH template plasmid	1 µl	< 1,000 ng
Milli-Q H20	9 µl	

MMut1_F (79C): CCTTCTGGTAGCTGAACCTGCAAAAGCGCGGCACCCGTCCGCTG

MMut1_R (79C): CAGGTTCAGCTACCAGAAGGTGACGACGCCCGTGACCACCGC

MMut2_F (79C): CTTCGTGCCCCGCTAGCAGGGGCCGGGCAG

MMut2_R (80C): CCTGCTAGCGGGGCACGAAGCCCGCGCCCAC

MMut3_F (79C): CGGTGCTGATCTAGTCGCAGGTCATTCTGTGTTTCGGGGTGCCCTTCGCG

MMut3_R (80C): CCTGCGACTAGATCAGCACCGACGACGGGTCCATGCCCAGC

DMut1_F (79C): GGCGCTGCAAAACACCTAGACCGAGCTGCAAGCCCTGCAACTCCAG

DMut1_R (80C): CAGGGCTTGCAGCTCGGTCTAGGTGTTTTGCAGGCGCTGGACGCTC

DMut2_F (79C): GCGCCTTCTAGCAGAACACGCCGACCGACCCCAACACCG

DMut2_R (80C): CGGTCGGCGTGTTCTGCTAGAAGGCGCGCATCTGCCACTGGTACTTCTCGATGATG

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
25–35 Cycles	98°C	10 seconds
	67°C	20 seconds
	72°C	1:40
Final Extension	72°C	2 minutes
Hold	4°C	Inf

Mutagenesis Screen 7-16-14

Design of a screen for the single base substitution mutations of the two Hell cell genes. This protocol works off of the PCR-CTPP method (Hamajima et al., 2000). The forward primer is to the unmutated portion of the gene and should bind to all plasmids. The reverse primers have the mutation at the 3' end, which should greatly decrease binding to non-mutated plasmids. Expect the mutation to produce a 250, 400, or 950 bp PCR product for M1, M2, or M3 respectively. Protocol may require modification of number of cycles or Tm to get no amplification for control DpsMP and MntH plasmids.

Component	Volume	Final Concentration
Amplitaq Gold 2X Master Mix	10 µl	1X
10 µM Forward/R verification mix	1 µl	1 µM
Plasmid	0.5 µl	< 1,000 ng
Milli-Q H20	8.5 µl	

Mscreen_f (62C): GATTCAGAACCTCAGCGCCA

M1screen_r (58C): CGCTTTTGCAGGTTCAGCT

M2screen_r (52C): GCCCGGCCCCTGCT

M3screen_r (60.6C): AACACAGAATGACCTGCGACT

Dscreen_f (62C): GTTTCCAGCCTGCTCCTCG

D1screen_r (56C):GGCTTGCAGCTCGGTCT

D2screen_r (56C): GGTCGGCGTGTTCTGCT

Primer mixes of Mscreen_f+[M1, M2, or M3]screen_r; or Dscreen_f+[D1 or D2]screen_r

Tested negative controls of primer mixes with DpsMP and MntH unmutated plasmids. Also tested with the cleaned product of each mutation PCR.

STEP	TEMP	TIME
Initial Denaturation	95°C	9 minutes
30 Cycles	95°C	45 seconds
Anneal	56°C	30 seconds
Extend	72°C	55 seconds
Final Extension	72°C	5 minutes
Hold	4°C	Inf

Ref:

Hamajima, N., Saito, T., Matsuo, K., Kozaki, K.-i., Takahashi, T. and Tajima, K. (2000), Polymerase Chain Reaction with Confronting Two-pair Primers for Polymorphism Genotyping. Cancer Science, 91: 865–868.

Acid Base Assay 9-24-2012

1. Grow up 5ml cultures of negative control, recA, dps, sdaB (one each).
2. Allow cultures to grow up overnight.
3. Set up acid and base solutions first. Fill 5 tubes with 10ml fresh LB and 50ul amp. Label: ph3.54, ph4.60, ph6.93, ph9.07, ph9.50. The HCl and NaOH have been sterilized in 50ml tubes. They are above the gels in the cabinets. They must always be stored in separate secondary containers. Eye protection is recommended. Glasses are above where we keep tip boxes. Acids and bases can go down the drain, just make sure there is plenty of running water. Any acidic or basic LB can go in liquid waste.

pH	mL of 1M HCl (sterile)	mL of 1M NaOH (sterile)
3.54	2ml	0
4.60	160ul	0
6.93	0	0
9.07	0	120ul
9.50	0	190ul

4. Verify 5ml of each acidity with pH meter.

5. Set up acid base 96 well plate. Each well contains 180ul of appropriate pH media and 5ul of cells.

	1	2 ph3.54	3 ph4.60	4 ph6.93	5 ph9.07	6 ph9.50	7 ph3.54	8 ph4.60	9 ph6.93	10 ph9.07	11 ph9.50	12
A	Blank	recA, ph3.54	recA, ph4.60	recA, ph6.93	recA, ph9.07	recA, ph9.50	Neg, ph3.54	Neg, ph4.60	Neg, ph6.93	Neg, ph9.07	Neg, ph9.50	empty
B	Blank	recA, ph3.54	recA, ph4.60	recA, ph6.93	recA, ph9.07	recA, ph9.50	Neg, ph3.54	Neg, ph4.60	Neg, ph6.93	Neg, ph9.07	Neg, ph9.50	empty
C	Blank	recA, ph3.54	recA, ph4.60	recA, ph6.93	recA, ph9.07	recA, ph9.50	Neg, ph3.54	Neg, ph4.60	Neg, ph6.93	Neg, ph9.07	Neg, ph9.50	empty
D	Blank	recA, ph3.54	recA, ph4.60	recA, ph6.93	recA, ph9.07	recA, ph9.50	Neg, ph3.54	Neg, ph4.60	Neg, ph6.93	Neg, ph9.07	Neg, ph9.50	empty
E	Blank	sdaB, ph3.54	sdaB, ph4.60	sdaB, ph6.93	sdaB, ph9.07	sdaB, ph9.50	dps, ph3.54	dps, ph4.60	dps, ph6.93	dps, ph9.07	dps, ph9.50	empty
F	Blank	sdaB, ph3.54	sdaB, ph4.60	sdaB, ph6.93	sdaB, ph9.07	sdaB, ph9.50	dps, ph3.54	dps, ph4.60	dps, ph6.93	dps, ph9.07	dps, ph9.50	empty
G	Blank	sdaB, ph3.54	sdaB, ph4.60	sdaB, ph6.93	sdaB, ph9.07	sdaB, ph9.50	dps, ph3.54	dps, ph4.60	dps, ph6.93	dps, ph9.07	dps, ph9.50	empty
H	Blank	sdaB, ph3.54	sdaB, ph4.60	sdaB, ph6.93	sdaB, ph9.07	sdaB, ph9.50	dps, ph3.54	dps, ph4.60	dps, ph6.93	dps, ph9.07	dps, ph9.50	empty

To summarize, the different color regions are different genes. The easiest way to do this is to fill 180ul of media into the right columns with the multichannel. Then put in 5ul of right cells. Column 1 is a blank which is 180ul LB + amp. Columns 2 and 7 are ph3.54 media, Columns 3

and 8 are ph4.60, Columns 4 and 9 are ph6.93, Columns 5 and 10 are ph 9, and Columns 6 and 11 are ph9.50.

6. Load plate into spec. Setup the spec using the computer. There should be a template from before saved in the iGEM folder on desktop. Make sure to set the spec to 37oC. Take the OD at 600 every 5 minutes for 24 hours. Shake the plate for 1 minute between reads and for 1 minute before each read. You need to specify which wells are blank. Also, in order for data to be collected, you need to label the wells and give them a name.

7. Let the spec run for 24 hours. After the 24 hours, take the data off the computer. Use the vacuum waste to clean up each well, and toss the plate in biohazard.